

Rediscovery, redescription and identity of *Pristimantis nebulosus* (Henle, 1992), and description of a new terrestrial-breeding frog from montane rainforests of central Peru (Anura, Strabomantidae)

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Abstract

The taxonomic status of the strabomantid frog species *Pristimantis nebulosus* (Henle, 1992), originating from the southern Cordillera Azul in central Peru, is investigated based on examination of the holotype and its comparison with freshly collected topotypic material. Following current standards, we provide a redescription of the holotype. It is in a rather poor state and exhibits certain damages and preservation artifacts, conditions that have hampered an allocation of this nominal taxon to any known living population of *Pristimantis* in the past. Our detailed specimen-to-specimen comparison provided morphological evidence for the conspecificity of the holotype and freshly collected topotypes. Molecular phylogenetic analysis, based on the mitochondrial 16S gene fragment places *P. nebulosus* in the *P. conspicillatus* species group, being closely related to *P. bipunctatus* and an undescribed candidate species from the Cordillera de Carpish. From both, *P. nebulosus* differs by considerable divergence in the 16S gene (p-distance 4.1–6.2%). Based on the specimens available, we provide an updated diagnosis for *P. nebulosus*, compare it to other species in the *P. conspicillatus* group and describe its advertisement call. In addition, we describe and name the closely related candidate species from the Cordillera de Carpish. It is sister to *P. bipunctatus* and *P. nebulosus*, but differs from both mainly by its tuberculate dorsal skin (versus shagreen) and divergence in the 16S gene (3.3–4.1%). We briefly discuss cryptic species diversity in the *P. conspicillatus* and *P. danae* species groups and provide justification for the description of a singleton species.

Key Words

Amphibia, bioacoustics, Cordillera Azul, Cordillera de Carpish, molecular genetics, morphology, *Pristimantis conspicillatus* species group, systematics, taxonomy

Introduction

The genus *Pristimantis* in the anuran family Strabomantidae currently comprises 590 recognised species (AmphibiaWeb 2022). Species in the genus are distributed from Central America southwards to southern Brazil and northern Argentina (Frost 2021), occupying a great variety of different biomes in lowland and montane areas. At present, 15 species groups are recognised in the genus

(Padiál et al. 2014; Gonzáles-Durán et al. 2017; Páez and Ron 2019; Chávez et al. 2021; Zumel et al. 2021), but for a majority of species only sparse data are available and these, therefore, remained unassigned to any group (Padiál et al. 2014). Furthermore, several studies indicated that species diversity in the genus is considerably underestimated by the number of names established, and demonstrated that resolving the taxonomy in this group might be a complex task because of intra-specific polymorphism

on the one hand and superficial crypsis on the other (e.g. Elmer and Cannatella 2008; Padial and De la Riva 2009; Padial et al. 2009; Ortega-Andrade et al. 2015; Lehr et al. 2017; Páez and Ron 2019). Nevertheless, the integrative application of molecular genetics, in combination with morphology and bioacoustics, has led to a continuous description of new *Pristimantis* species at an unrestrained rate (e.g. Hutter and Guayasamin 2015; Páez and Ron 2019; Moravec et al. 2020; Carrión-Olmedo and Ron 2021; Ortega-Andrade et al. 2021).

In contrast to the accelerating number of species recognised in the genus *Pristimantis*, knowledge about many nominal species is poor and sometimes species are only known from the type specimens and have subsequently never been allocated to any known living population. One of those species is *Pristimantis nebulosus* (Henle, 1992). The original description of this taxon (as *Eleutherodactylus nebulosus*) was based on a single specimen collected in April 1979 by Rainer Schulte in the southern Cordillera Azul in central Peru, and the species was allocated to the *E. fitzingeri* species group (sensu Lynch 1976) according to a few morphological characters, such as smooth belly, first finger longer than second and tympanum distinct (Henle 1992). Subsequently, Lynch (1996) established a replacement name for this taxon (*Eleutherodactylus caliginosus*), as Henle's (1992) name constituted a junior homonym of *Syrrhophus nebulosus* Taylor, 1943 (actually a secondary homonym, not a primary homonym, as stated by Lynch 1996; see ICZN 1999: Article 57.3), a taxon periodically considered to be synonymous with *Eleutherodactylus pipilans* (Taylor, 1940) and now considered to represent a valid Central American species, *Eleutherodactylus nebulosus* (see Grünwald et al. 2021). The subsequent transfer of many species of *Eleutherodactylus* to other genera (Hedges et al. 2008) and the allocation of Henle's taxon to *Pristimantis* again reestablished the species name *nebulosus*, as a homonymy was no longer in place (ICZN 1999: Article 59.4; Duellman and Lehr 2009).

Since its description, *Pristimantis nebulosus* remained an enigmatic taxon that apparently had never been recollected and was rarely mentioned in the literature. Lynch (1996) and Lynch and Duellman (1997) placed it in the *P. conspicillatus* species group (as *E. caliginosus*) and, subsequently, it was listed in systematic accounts without allocation to any species group (Heinicke et al. 2007; Hedges et al. 2008; Padial et al. 2014). Given that its original description (Henle 1992) is barely detailed and partly inadequate, covering less than two pages including a figure of the preserved holotype, and the fact that the type specimen is in a rather poor state of preservation, its identity and relationships remained obscure. Duellman and Lehr (2009), in their monograph on Peruvian terrestrial-breeding frogs, mentioned that, despite examination of the holotype, they were unable to associate the nominal taxon with any known population of *Pristimantis*.

During fieldwork in November 2019 in central Peru, we collected *Pristimantis* frogs in the Departamentos Huánuco and Ucayali. Among the investigated sites were the

Cordillera de Carpish and the southern parts of the Cordillera Azul in central Peru (see Castillo-Urbina et al. 2021), the latter including the type locality of *P. nebulosus*. Some of the specimens collected at the *P. nebulosus* type locality appeared to represent members of the *P. conspicillatus* species group and, superficially, were in agreement with the description of *P. nebulosus*. The present study aims at the clarification of the taxonomic status of these newly collected specimens and an evaluation of the identity of *P. nebulosus*, as well as the description of a closely related new species from the Cordillera de Carpish.

Material and methods

Fieldwork and voucher specimens

Fieldwork was conducted in November 2019 in different areas of the Departamentos Huánuco and Ucayali in central Peru. Specimens were observed and collected during opportunistic searching at night using torches and headlamps, often guided by the sounds emitted by calling males. Geographic position was recorded using handheld GPS receivers set to WGS84 datum.

Collected specimens were euthanised using an overdose of 5% lidocaine gel applied on ventral surfaces of the individuals (McDiarmid 1994). Tissue samples (thigh muscle and tongue pieces) were cut prior to fixation and stored in 99% ethanol, while specimens were fixed using 96% ethanol and subsequently stored in 70% ethanol. Specimens were deposited at the herpetological collection of the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM), Lima, Peru. FGZC refers to Frank Glaw field numbers, KU refers to University of Kansas, Museum of Natural History, Division of Herpetology, Lawrence, Kansas, USA, and ZFMK refers to Zoologisches Forschungsmuseum A. Koenig, Bonn, Germany.

External morphology

Morphometric measurements (in millimetres) were taken by JK with a digital calliper to the nearest 0.1 mm. Measurements taken and used throughout the text are: **SVL**, snout–vent length; **TL**, tibia length; **HW**, head width (at level of angle of jaws); **HL**, head length (from posterior margin of lower jaw to tip of snout); **IOD**, interorbital distance; **ED**, horizontal eye diameter; **E–N**, eye–nostril distance (straight line distance between anterior corner of orbit and posterior margin of external nares); **TD**, horizontal tympanum diameter; **HandL**, hand length (from proximal border of outer metacarpal tubercle to tip of third finger); **FootL**, foot length (from proximal border of inner metatarsal tubercle to tip of fourth toe). Fingers and toes are numbered preaxially to postaxially from I–IV and I–V, respectively. Lengths of fingers I and II were determined by adpressing the fingers against each other. For character state definitions, we followed Duellman

and Lehr (2009). Description and diagnostic schemes follow Padial et al. (2016). Colouration in life was described based on digital photographs.

Bioacoustics

Vocalisations in the field were recorded using a Olympus LS-05 digital recorder with built-in microphones at 44.1 KHz and 16-bit resolution and saved as uncompressed wave format. Recordings were re-sampled at 22.05 kHz and 32-bit resolution and computer-analysed using the software Cool Edit Pro 2.0. We obtained frequency information through Fast Fourier Transformation (FFT; width 1024 points) at Hanning window function. Spectrograms were drawn with Blackman window function at 256 bands resolution. Sensitive filtering was applied to remove background sounds, applied only to frequencies outside the prevalent bandwidths of calls. Temporal measurements are summarised as range with mean \pm standard deviation in parentheses. Description and terminology follow the call-centred scheme of Köhler et al. (2017).

Taxon sampling

Our genetic analysis aimed at identifying lineage divergence among focal lineages of *Pristimantis*. For representative taxon sampling, we largely followed an approach as described by Castillo-Urbina et al. (2021). We compared sequences of three of our collected specimens of the focal species with available sequences of the 16S rRNA gene of *Pristimantis*. For this, we first blasted the sequences of the new samples against GenBank and downloaded sequences with an identity $> 89\%$. Next, we manually searched for sequences of species that are currently placed in the *P. conspicillatus* species group, plus representatives of the *P. danae* species group (sensu Padial et al. 2014), as some of the latter group share superficial morphological similarities with species in the *P. conspicillatus* group. In addition, we selected a small set of sequences of representatives assigned to the *P. lacrimosus* and *P. ridens* species groups, as some members occur in the same general area as the focal lineages. For the *P. conspicillatus* and *P. danae* species groups, we further added seven new samples collected by us at different localities in central Peru. We unsuccessfully tried to obtain a DNA sequence from a small piece of thigh muscle from the holotype of *E. nebulosus* (ZFMK 27634) using the standard protocol described below. A sequence of *Yunganastes phuvicanorus* was included as outgroup. For details of used samples, see Appendix 1.

Molecular analyses

We sequenced a DNA fragment of the mitochondrial 16S rRNA gene (16S) from tissue samples of newly collected specimens. In brief, DNA was extracted using a

standard salt extraction protocol, Polymerase Chain Reaction (PCR) carried out with primers 16SAr-L (5'-CG-CCTGTTTATCAAAAACAT-3') and 16SBr-H (5'-CCG-GTCTGAACTCAGATCACGT-3') (Palumbi et al. 1991) and the PCR products then directly sequenced on automated DNA sequencers by LGC Genomics (Berlin, Germany). All new DNA sequences were submitted to GenBank (accession numbers ON710980–ON710989). MEGA7 (Kumar et al. 2016) was used to align sequences to reference sequences of other *Pristimantis* downloaded from GenBank using the Muscle algorithm (total resulting alignment length 584 bp), and to identify the GTR+I+G substitution model as best fitting the data set under the Bayesian Information Criterion. We used this substitution model to infer a Maximum Likelihood (ML) tree in RAxML v8 (Stamatakis 2014) as implemented in raxmlGUI v2.0 (Edler et al. 2021), assessing node support with 500 thorough non-parametric ML bootstrap replicates. To quantify genetic divergences, we calculated uncorrected pairwise distances among the sequences (p-distances) in MEGA7.

Results and conclusions

The holotype of *Eleutherodactylus nebulosus* Henle, 1992

We examined the holotype of *Eleutherodactylus nebulosus* (ZFMK 27634) in detail. As former authors already mentioned (Duellman and Lehr 2009), we confirm that the specimen is in a very poor state. The body is rather soft, whereas the head and distal parts of fingers and toes are hardened and completely desiccated. We speculate that the specimen once was almost completely dry and then after immersion in preservation liquid partly softened. Such phenomena may occur if, for example, the collected individual died in the collection bag due to heat and is transferred to preservation liquid a certain time after without proper fixation. Judging from the figure provided by Henle (1992), the holotype was already in this poor condition at the time of its description. This fact may have resulted in the description of preservation artifacts erroneously considered to represent morphological traits by Henle (1992). To somehow quantify the condition of the type specimen, we in the following list the most obvious artifacts and damages: (1) vertebral column broken close to urostyle, resulting in body being longitudinally compressed, with virtually shorter SVL; urostyle bones protruding dorsally; (2) head desiccated, lacking almost all fleshy parts, dorsolaterally compressed and squeezed, resulting in virtually greater HW; (3) distal parts of fingers completely desiccated; and (4) distal parts of toes completely desiccated (Fig. 1).

We here provide a redescription of the holotype of *Eleutherodactylus nebulosus* Henle, 1992 (ZFMK 27634) using current standards, considering that several character descriptions are to be treated with some caution or are not accessible (NA) due to the poor state of preservation.

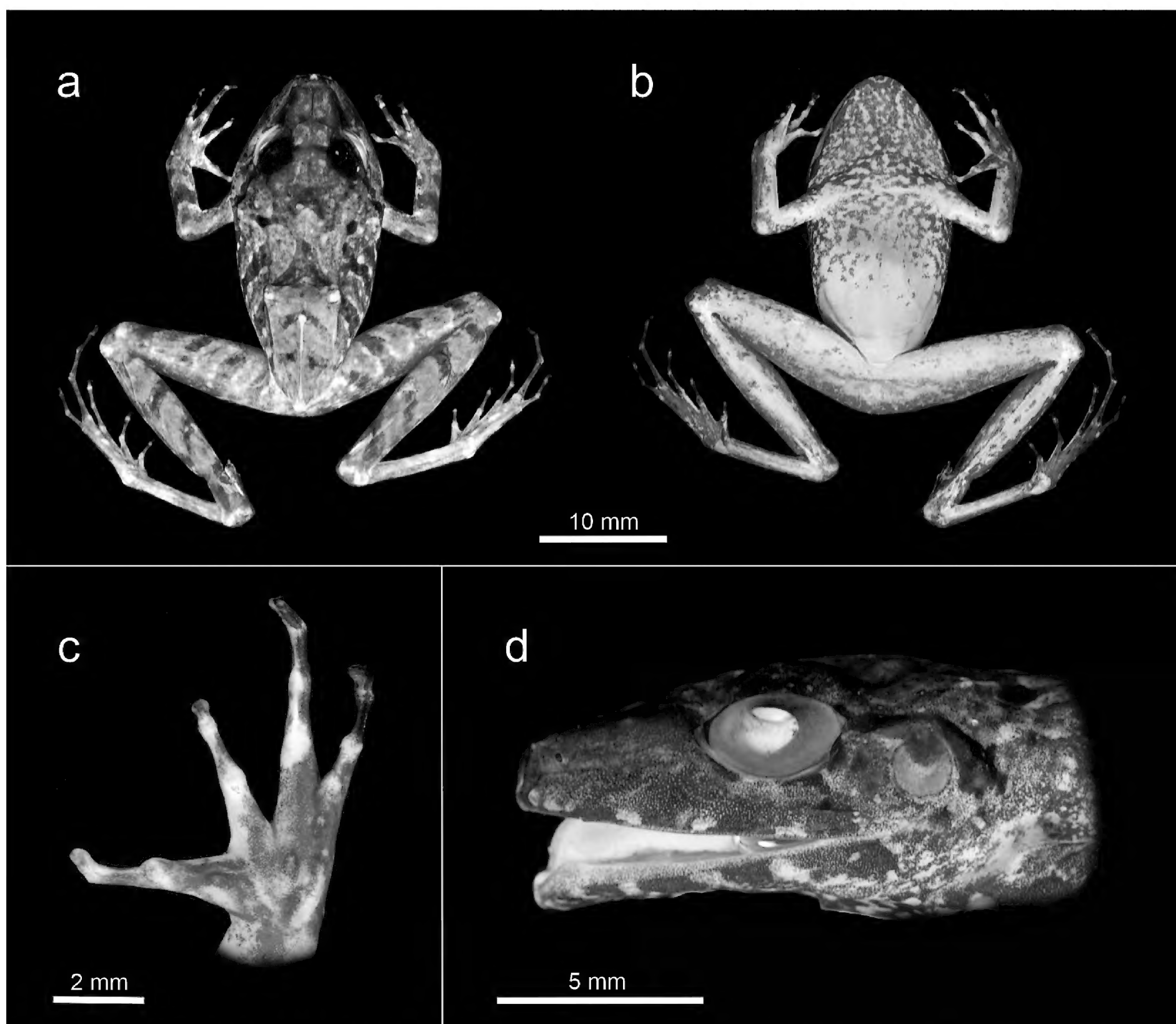


Figure 1. Preserved holotype of *Eleutherodactylus nebulosus* Henle, 1992 (ZFMK 27634) in **a.** dorsal and **b.** ventral views; **c.** Palmar surface of left hand; **d.** Head in lateral profile. Note that due to the poor state of preservation and damage, certain characters appear to be altered, including body shape, finger length, finger discs, and head shape.

Eleutherodactylus nebulosus Henle, 1992

Redescription of the holotype. An adult male (Fig. 1) with subgular vocal sac and vocal slits. SVL 28.6 mm (for additional measurements see Table 1). Head slightly longer than wide (HL/HW = 1.1); snout shape NA; nostrils oriented posterolaterally; canthus rostralis straight in dorsal view, sharp in profile; loreal region very slightly concave; lips not flared; upper eyelid without tubercles; cranial crests absent. Supratympanic fold prominent, long, curved, surrounding the upper tympanic membrane; tympanic membrane and annulus distinct; tympanic membrane nearly round on the left side, slightly higher than long on right side, its length less than half the eye diameter; two prominent postrictal tubercles, conical. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, ovate, separated by distance equal to six times diameter of a choana; dentigerous process

of vomers prominent, triangular in shape, not in contact, oblique, situated posteromedial to choanae; vocal sac subgular, vocal slits placed posterolaterally. Skin of dorsal surfaces shagreen, with few scattered low tubercles; skin on throat, chest and belly weakly areolate, almost smooth; occipital folds absent; dorsolateral folds present, low, formed by a discontinuous row of anastomosed granules; discoidal fold conspicuous. Arm without ulnar tubercles; palmar tubercle bifid, relatively flat, equal to elongate thenar tubercle; few supernumerary tubercles present, low, round, smaller than subarticular tubercles; subarticular tubercles prominent, subconical; finger tips enlarged (NA completely due to desiccation and shrinking); fingers without lateral fringes; basal webbing between fingers II and III; relative length of fingers $III > IV > II \leq I$; traces of a single non-spinous nuptial pad on dorsal surface thumb. Toes long and slender (FootL 55% of SVL); heel and tarsus lacking tubercles; tarsal fold NA; inner metatarsal tubercle ovate, flat, larger than

Table 1. Morphological measurements (in mm) of specimens of *Pristimantis nebulosus* from Abra La Divisoria, Departamento Huánuco, Peru, 1650 m a.s.l. For abbreviations, see Material and methods. Values in parentheses to be considered with reservations due to the poor condition of the holotype specimen.

	ZFMK 27643 holotype	MUSM 40298 topotype	MUSM 40299 topotype	MUSM 40326 topotype
	male	male	male	juvenile
SVL	(28.6)	29.1	27.7	19.7
TL	18.7	18.7	17.6	11.4
HL	(11.8)	11.6	10.9	8.6
HW	(10.6)	10.1	9.6	7.2
IOD	3.1	3.3	3.1	2.4
ED	(4.0)	4.1	4.4	2.9
TD	1.6	1.6	1.6	1.1
E–N	(3.5)	3.4	3.4	2.5
HandL	(8.3)	8.3	8.0	4.8
FootL	(15.9)	15.8	15.6	9.7

outer; outer metatarsal tubercle round, flat; supernumerary tubercles NA; subarticular tubercles present (but strongly desiccated); lateral fringes on toes NA, basal toe webbing present; toe tips NA (but T-shaped phalanges visible, indicating expanded toe tips); ungual flap and circumferential grooves NA; relative length of toes IV > V > III > II > I; toe V reaching proximal level of penultimate subarticular tubercle of toe IV. Tibiotarsal articulation reaches far beyond tip of snout when hind limb flexed parallel to axis of body; heels broadly overlapping when hind limbs flexed perpendicular to axis of body.

After 42 years in preservative (Fig. 1), the dorsal surfaces are overall tan to brown of different shades, with dark brown chevrons outlined with cream; arms and legs are barred with brown bars the same colour as those of dorsum and the same colour applies to vertical bars on the flanks, which are also outlined with cream; dark brown interorbital bar; canthus rostralis darker than surrounding skin; triangular cream coloured fleck on tip of snout; supratympanic fold and a pair of occipital spots black; posterior surfaces of thighs brown, with fine scattered irregular cream spots; chest and belly greyish-white with some scattered irregular brown flecking on chest; throat cream-coloured, but densely mottled with fine brown to grey spots and flecking which (like those on chest) are rather distinct and contrasted; lower and upper lips brown and barred with cream; soles of hands and feet brown with irregular cream flecking.

Our examination of the type specimen revealed the following discrepancies to the original description: Henle (1992) described the first finger being longer than the second. Although this appears to be correct at first view (see Fig. 1), it is to be mentioned that the tip of finger II is almost completely desiccated and thus the finger apparently lost some of its total length. However, even at this state of preservation, when fingers I and II are adpressed against each other, they are about equal in length. At most, we

could state that finger I is only slightly longer than finger II. Henle (1992) described the terminal finger discs as being only slightly expanded. As stated above, distal fingers of the holotype are completely desiccated and apparently were already during the time of the species description (Henle 1992: fig. 2). As a consequence, finger discs are barely obvious, but when viewed with magnification, T-shaped terminal phalanges are clearly visible, indicating the probable former presence of expanded finger discs. The original description (Henle 1992) stated that an outer metatarsal tubercle is lacking. The desiccation event apparently resulted in less distinct and less elevated tubercles on hands and feet (including subarticular tubercles) in the holotype, but with strong magnification (> 20×), skin structures become evident which likely once represented a flat outer metatarsal tubercle. Moreover, Henle (1992) described the tympanum as being slightly higher than long, a state we were able to confirm only for the right body side of the holotype, whereas it exhibits a nearly round tympanum on the left body side (Fig. 1). We reject Henle’s (1992) statement that toes exhibit webbing of about one fourth of their length, as only basal webbing is present between the toes of the holotype. The supratympanic fold has been described as weakly developed by Henle (1992), but it is actually rather distinct in the holotype. Due to the condition of the type specimen, we were unable to confirm or reject Henle’s (1992) characterisation of the snout shape. As mentioned above, fleshy parts are almost completely lacking at the anterior head of the holotype and the shape of the head in its current condition seems to be basically represented by the skull shape. Moreover, the head shape has likely been altered by its dorsoventral compression and squeezing.

New topotypic specimens

In the the original description of *P. nebulosus*, the type locality is given as “Departamento Huanuco, Cordillera Azul, Paß der Carretera Central auf 1650 m NN” (Henle 1992), which translates to Departamento Huánuco, Cordillera Azul, pass of the Carretera Central at 1650 m above sea level. Henle (1992), in his itinerary, called this same locality “Divisoria, Cordillera Azul (1650)”. During our fieldtrip, we searched the surroundings of a spot locally called ‘Abra La Divisoria’ (9.20017°S, 75.79531°W), a pass of the national road 5N at 1650 m a.s.l. at the border of the Departamentos Huánuco and Ucayali, where a small restaurant and fuel station are located. This locality is not to be confused with the small village called ‘Divisoria’, located few kilometres eastwards and downhill along the same road, situated already in Departamento Ucayali. According to the concordant elevation, presence of a road pass and the name of the locality, we have no doubts that ‘Abra La Divisoria’ actually constitutes the type locality of *P. nebulosus*.

At Abra La Divisoria, we collected two adult males (MUSM 40298–40299, FGZC 6253–6254; coll. 8

November 2019) and one juvenile (MUSM 40326, FGZC 6281; coll. 10 November 2019) of a species of *Pristimantis* which, due to their general appearance, could be identified as members of either the *P. conspicillatus* or the *P. danae* species group (sensu Padial et al. 2014). These three specimens had identical sequences of the 16S gene (see below for molecular relationships) and, thus, are to be considered conspecific. Despite unclear relationships, *P. nebulosus* has been associated with the *P. conspicillatus* species group by Lynch (1996) and Lynch and Duellman (1997), which led us to hypothesise that the newly collected specimens might constitute a rediscovery of this enigmatic species. Given that the original species description (Henle 1992) is barely helpful for proper identification, we compared our collected specimens with the holotype of *E. nebulosus* in detail. At first superficial examination, the holotype seemed to differ from our specimens by a stouter body shape and broader head. However, as described above, the poor state of preservation and certain damage apparently are reasons

for this particular appearance. Our detailed comparison of morphological characters did not reveal any diagnostic differences between the holotype and our specimens which are not explainable by damage, preservation artifacts, or intra-specific variation.

Our collected adult male specimens (Figs 2, 3) generally agree morphologically with the male holotype of *E. nebulosus* as described above in all diagnostic characters, namely: similar size and overall proportions (see Table 1 for measurements); skin on dorsum shagreen with few scattered small tubercles, skin of throat and venter weakly areolate; discoidal fold conspicuous; indistinct, low dorsolateral fold; tympanum and tympanic annulus distinct, its diameter slightly less than half of eye diameter, nearly round; supratympanic fold prominent, curved, surrounding upper tympanum; two prominent conical postictal tubercles; occipital folds absent; head slightly longer than wide; canthus rostralis straight in dorsal view, sharp in profile; dentigerous process of vomers prominent, triangular in outline, oblique, situated

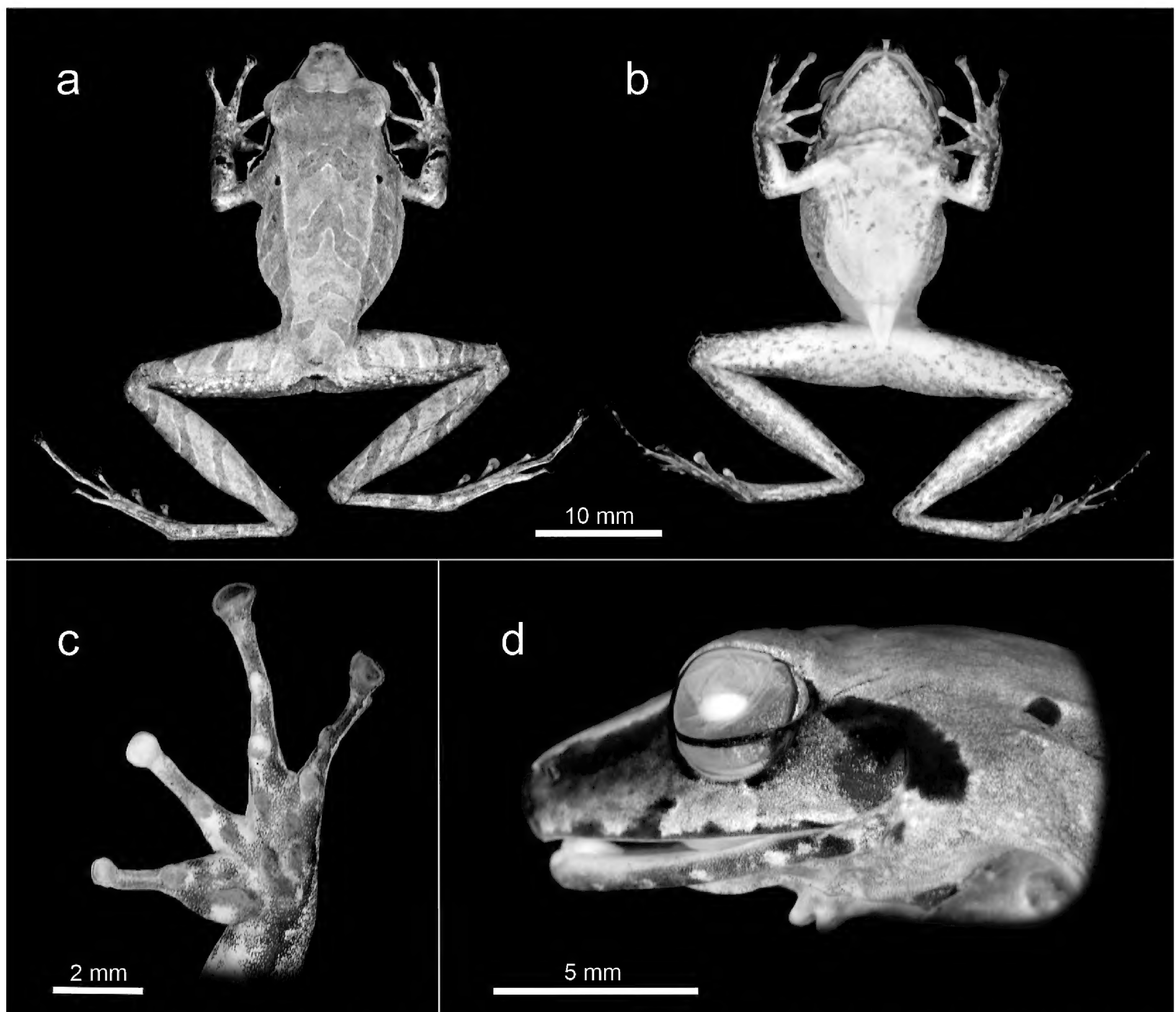


Figure 2. Preserved topotypic male of *Pristimantis nebulosus* (MUSM 40299, FGZC 6254) from Abra La Divisoria, Cordillera Azul, 1650 m a.s.l., Peru, in **a.** dorsal, and **b.** ventral views; **c.** Palmar surface of left hand; **d.** Head in lateral profile.

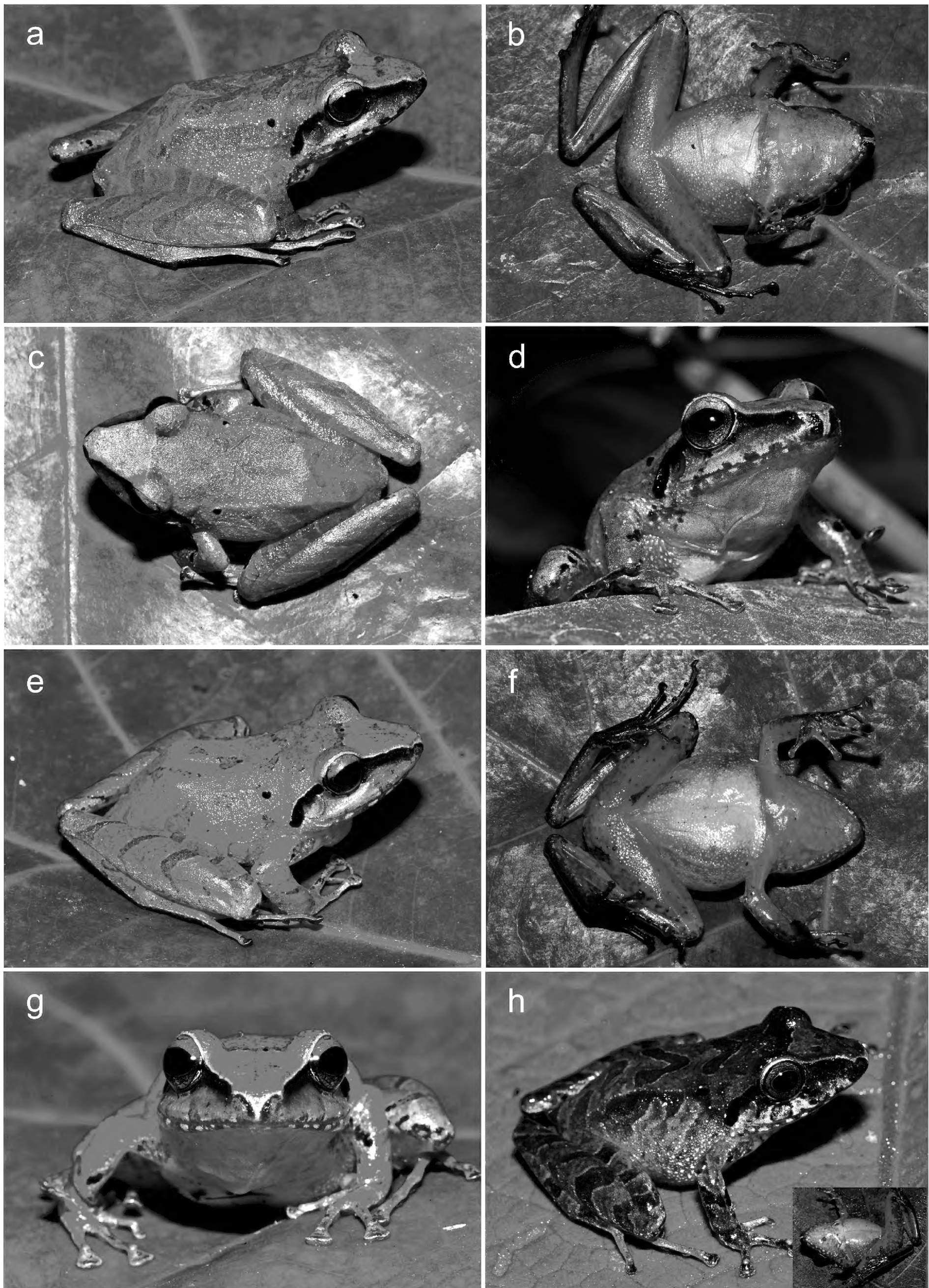


Figure 3. Topotypic *Pristimantis nebulosus* in life: Adult male (MUSM 40299) in **a.** dorsolateral, **b.** ventral, **c.** dorsal, and **d.** frontolateral views; adult male (MUSM 40298) in **e.** dorsolateral, **f.** ventral, and **g.** frontal views; **h.** Juvenile (MUSM 40326) in dorsolateral view (inset photo showing ventral view of same individual). Not to scale.

posteromedial to choanae; first finger only very slightly longer than second, almost equal in length; lateral fringes on fingers absent, basal webbing between fingers II and III; subarticular tubercles prominent, conical; palmar tubercle bifid, thenar tubercle ovoid; terminal finger discs expanded; toes long and slender, basal webbing between toes; toe V slightly longer than toe III; inner tarsal tubercle elongated, prominent; outer metatarsal tubercle inconspicuous, ovoid; terminal toe discs expanded; dorsum with dark chevron-shaped markings, outlined with cream; a pair of occipital black spots; dark interorbital bar; flanks with dark diagonal bands and upper surfaces of arms and thighs with dark crossbands, all outlined with cream; triangular cream-coloured fleck on tip of snout; throat with dark spotting and mottling, chest and belly cream with some scattered dark flecks anteriorly; ventral surfaces of thighs with dark spotting; posterior surfaces of thighs dark with fine cream spotting; upper and lower lip dark with irregular cream spots and bars; broad black supratympanic stripe.

Despite this congruence in character states, some observations are in need of further comments. The shape of terminal discs on fingers is almost undiscoverable in the holotype due to complete desiccation and shrinking. However, given the presence of clearly T-shaped terminal phalanges in the type specimen, we are convinced that it actually exhibited distinctly expanded finger tips as present in our fresh material. The ground colouration of the preserved holotype is considerably darker and more brownish compared to our adult males (predominantly greyish with some tan brown to reddish tint in preservative). This is particularly obvious with respect to the less contrasting pattern of dark spots and mottling on throat and chest (compare Figs 1, 2). This ventral pattern is brown, rather contrasting and conspicuous in the holotype, while it is grey and appears less dense and less contrasting in our specimens. Nevertheless, the general pattern of ventral markings is the same among all specimens, with the throat most densely mottled, mottling and flecking present on chest and more widely spaced flecking on the anterior belly. Overall, the holotype is distinctly darker coloured and brownish when compared to our preserved greyish specimens, but the more contrasting pattern on the dorsum and venter may most likely be explained as a result of the preservation method used for the holotype (which remains unknown). Although only few specimens were available, variation is particularly evident in the colour pattern. The three specimens collected by us differ in the extent and shape of dark dorsal markings. Remarkably, the juvenile specimen (MUSM 40326) is considerably darker in ground colour compared to the two adult males and has a more contrasting pattern (Fig. 3), thus very strongly resembling the colour and pattern of the holotype. Another difference constitutes the presence of a broad dark canthal stripe in our specimens, not evident in the holotype. Given that the canthal region in the holotype is slightly darker coloured compared to the surrounding skin, particularly on the right side, we

suspect that this pattern simply faded. This assumption might appear unjustified, as otherwise the colour pattern of the holotype is more contrasting compared to the freshly collected material, but obviously the head of the holotype body suffered most from desiccation which might have also promoted colour fading to a greater extent.

In summary, our morphological comparison revealed overall congruence in character states between the holotype and newly collected topotypes. Differences observed can either be explained by damage and preservation artifacts, or intra-specific variation, respectively. We finally conclude that the specimens collected by us at the type locality of *P. nebulosus* are conspecific with the respective holotype.

Identity of *Pristimantis nebulosus*

Pristimantis nebulosus (Henle, 1992)

Relationships. The Maximum Likelihood tree, based on an alignment of 584 bp of the 16S gene, is insufficient to establish a reliable hypothesis of phylogenetic relationships among the *Pristimantis* samples included (Fig. 4). The *P. conspicillatus* species group is recovered paraphyletic with respect to the sample of *P. gaigeae* from Panama, a member of the *P. conspicillatus* group sensu Hedges et al. (2008) and Padial et al. (2014). Together with species representing the *P. lacrimosus*, *P. ridens* and *P. danae* species groups, *P. gaigeae* is placed sister to the remaining species of the *P. conspicillatus* group. Within the *P. conspicillatus* group, most relationships are not resolved and basal nodes did not receive significant support. However, the three specimens of the focal *P. nebulosus* are recovered with high support (bootstrap value 98%) as members of a clade containing nominal *P. bipunctatus* and an undescribed candidate species, with *P. nebulosus* placed sister to nominal *P. bipunctatus* (without significant support; 36%). The clade containing *P. nebulosus*, *P. bipunctatus*, and the candidate species are recovered sister (76%) to a clade containing two samples of *P. skydmainos* (a close relationship of *P. bipunctatus* and *P. skydmainos* has already been revealed by Padial et al. 2014). Our *P. nebulosus* specimens had uncorrected p-distances in the studied 16S gene fragment of 5.4–6.2% to *P. bipunctatus* (with the greatest distance of 6.2% referring to the *P. bipunctatus* holotype KU291638) and 4.1% to the mentioned candidate species (Table 2). P-distances to other species in the *P. conspicillatus* species group range from 5.8–17.8%.

Diagnosis. Based on the holotype and the newly collected material, we provide the following updated diagnosis for *Pristimantis nebulosus* (Henle, 1992): a medium-sized species of the *Pristimantis conspicillatus* species group (based on molecular relationships and morphological similarity), with 27.7–29.1 mm SVL in adult males (n = 3), characterised by: (1) skin on dorsum shagreen, with few scattered small tubercles; flanks shagreen;

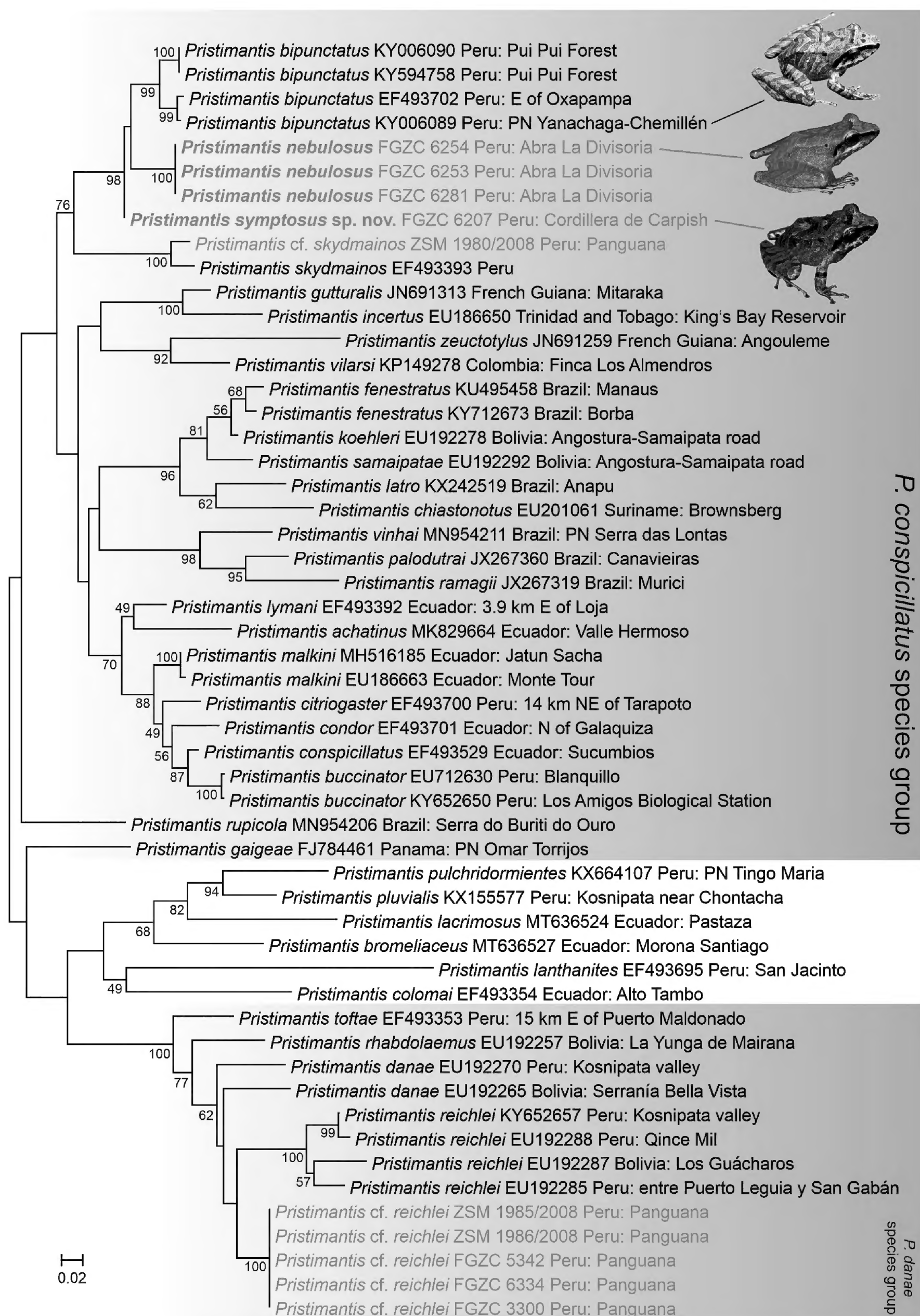


Figure 4. Maximum Likelihood tree inferred from a 584 bp fragment of the mitochondrial 16S gene of *Pristimantis* species. Values at nodes are bootstrap proportions in percent (values below 49% not shown). Newly obtained sequences for this study are in red font. For other samples used, species names are followed by GenBank accession number and locality. *Yunganastes pluvicanorus* was used as outgroup (not shown for graphic reasons only). Inset photos depict *Pristimantis bipunctatus* (MUSM 31120), *P. nebulosus* (MUSM 40299, FGZC 6254) and *P. symptosus* sp. nov. (MUSM 40256, FGZC 6207) in life.

Table 2. Estimates of uncorrected p-distances in a 491 bp fragment of the mitochondrial 16S gene among selected samples of the *Pristimantis conspicillatus* species group, calculated with MEGA7 (all ambiguous positions were removed for each sequence pair).

	FGZC 6253	FGZC 6254	FGZC 6281	FGZC 6207	KU 291638	MUSM 31179	MUSM 31120
<i>P. nebulosus</i> FGZC 6253 (Abra La Divisoria)							
<i>P. nebulosus</i> FGZC 6254 (Abra La Divisoria)	0.000						
<i>P. nebulosus</i> FGZC 6281 (Abra La Divisoria)	0.000	0.000					
<i>P. symptosus</i> FGZC 6207 (Cordillera de Carpish)	0.041	0.041	0.041				
<i>P. bipunctatus</i> KU 291638 (E of Oxapampa)	0.062	0.062	0.062	0.041			
<i>P. bipunctatus</i> MUSM 31179 (Pui Pui Forest)	0.054	0.054	0.054	0.033	0.037		
<i>P. bipunctatus</i> MUSM 31120 (Yanachaga Chemillén)	0.058	0.058	0.058	0.039	0.006	0.031	
<i>P. bipunctatus</i> NMP6V/75062 (Pui Pui Forest)	0.054	0.054	0.054	0.033	0.037	0.000	0.031

throat, chest and venter weakly areolate, smooth only in the middle and anterior part; posterior surfaces of limbs smooth; discoidal fold conspicuous; dorsolateral folds present, but low, partly interrupted; dorsal folds absent; two prominent postrictal conical tubercles present; upper eyelid lacking tubercles and granules; (2) tympanic membrane and annulus distinct, nearly round, their length slightly less than half of eye diameter; supratympanic fold long, prominent, curved, surrounding upper tympanum; (3) head slightly longer than wide; snout subacuminate in dorsal view, rounded in lateral profile; canthus rostralis straight in dorsal view, sharp in profile; (4) cranial crests absent; (5) dentigerous process of vomers large, triangular in outline, oblique, situated posteromedial to choanae; (6) males with vocal slits, single subgular vocal sac, and nuptial asperities on dorsal surface of thenar tubercle; (7) hands with long and slender fingers, first finger only very slightly longer than second; subarticular tubercles subconical, prominent; supernumerary tubercles round, smaller than subarticular tubercles; palmar tubercle bifid; thenar tubercle elongated; terminal discs of inner two fingers enlarged and round, those of external fingers enlarged, ovate to triangular, about twice the width of digit proximal to disc; circumferential grooves conspicuous, ungual flap slightly indented; lateral fringes and keels on fingers absent; basal webbing between fingers II and III; (8) ulnar tubercles absent; (9) tubercles on heel and tarsus absent, tarsal fold present, narrow and oblique; (10) inner metatarsal tubercle ovate, prominent; outer metatarsal tubercle round, flat, inconspicuous; supernumerary tubercles absent; (11) toes long and slender (FootL 54–56% SVL); narrow lateral fringes weakly expressed, basal toe webbing present; toe V reaching mid-level of penultimate subarticular tubercle of toe IV; toe V slightly longer than toe III; tips of toes rounded to slightly truncate, enlarged; circumferential grooves evident, ungual flap not indented; (12) in life, dorsal colouration overall brown of different shades (sometimes with orange to reddish tint), with dark brown chevrons or irregular flecks on dorsum, outlined with cream; dark brown bars on dorsal surfaces of arms and legs, outlined with cream; brown interorbital bar, at least anteriorly outlined with cream; a pair of black occipital spots; broad black supratympanic stripe; greyish-black canthal stripe; lips dark brown, barred with cream; tip of snout with triangular cream-coloured

fleck; belly yellowish-cream; throat yellow or yellowish-cream, mottled with grey spots; ventral surfaces of thighs and shanks orange brown to reddish-brown; posterior surface of thighs reddish-brown with small light spots; iris bronze, with some dark brown reticulation and a median reddish-brown streak; posterior iris periphery turquoise; bones white; (13) advertisement call consisting of a single pulsed note of 206–382 ms duration with a pulse rate of 156–174 pulses/second, emitted in short series (see below).

Comparison. Among the now 41 recognised species in the *P. conspicillatus* species group (Padial et al. 2014, 2016; de Oliveira et al. 2017, 2020; Taucce et al. 2020), 12 species, including *P. nebulosus*, exhibit dorsolateral folds (Duellman and Lehr 2009; Padial et al. 2016). Among these, *P. avicuporum* mainly differs by the presence of an interocular dermal fold, fingers bearing lateral fringes, and an upper eyelid with numerous tubercles (Duellman and Pramuk 1999; Duellman and Lehr 2009); *P. buccinator* mainly differs by continuous and prominent dorsolateral folds, an X-shaped or V-shaped dorsal fold and presence of an interorbital dermal ridge (Rodríguez 1994); *P. condor* mainly differs by larger male size (SVL 32.1–39.5 mm), finger I distinctly longer than finger II, lack of basal webbing between fingers II and III, and lack of basal webbing between toes (Lynch and Duellman 1980); *P. conspicillatus* mainly differs by finger I being distinctly longer than finger II, fingers with lateral keels, lack of basal webbing between fingers II and III, and posterior surfaces of thighs with orange spots in life (Duellman and Lehr 2009); *P. malkini* mainly differs by slightly larger male size (SVL 30.4–37.2 mm), finger I distinctly longer than finger II, and more extensive webbing between toes (Lynch 1980; Duellman and Lehr 2009); *P. meridionalis* mainly differs by finger I being shorter than finger II, dentigerous processes of vomers small and round, and fingers bearing lateral fringes (Duellman and Lehr 2009); *P. peruvianus* mainly differs by prominent and continuous dorsolateral folds, finger I distinctly longer than finger II, fingers with lateral fringes, and lack of basal webbing between toes (Köhler 2000; Padial and De la Riva 2009); *P. skydmainos* mainly differs by more prominent and continuous dorsolateral folds, finger I being shorter than finger II, and a black mid-dorsal tubercle (Flores and Rodríguez 1997; Padial and De la Riva 2005).

Superficially, *P. nebulosus* is similar to *P. iiap*, with which it shares similar adult male size, inconspicuous and partly interrupted dorsolateral folds, finger I only very slightly longer or equal in length to finger II, presence of postrostral tubercles, and similar colour pattern (Padial et al. 2016). However, *P. iiap* mainly differs by the lack of basal webbing between fingers II and III (present in *P. nebulosus*), lack of basal toe webbing (present), upper eyelid with small granules (absent), a copper-coloured iris in life (bronze), and advertisement call (Padial et al. 2016; see below). The closely related species *P. bipunctatus* also shares many characters with *P. nebulosus*, such as similar male size, dorsolateral folds present but inconspicuous, finger I about equal in length to finger II, upper eyelid lacking tubercles or granules, and similar colour pattern. However, it differs from *P. nebulosus* by the lack of basal webbing between fingers II and III (present), bluntly rounded snout in dorsal view (subacuminate), truncate in profile (rounded), basal webbing between toes IV and V (basal webbing between all toes) (Duellman and Hedges 2005; Duellman and Lehr 2009), and considerable divergence in the mitochondrial 16S gene. According to Duellman and Lehr (2009), *P. adistolus* is morphologically cryptic to *P. bipunctatus*. However, *P. adistolus* mainly differs from *P. nebulosus* by more prominent and continuous dorsolateral folds, lack of basal webbing between fingers, and finger I slightly shorter than finger II (Duellman and Hedges 2007). Individuals of the recently described species *P. pictus*, *P. pluvian* and *P. moa* from Amazonian Brazil may sometimes exhibit weakly developed dorsolateral folds, but all differ from *P. nebulosus* by smooth mid-venter (versus areolate) and advertisement call (de Oliveira et al. 2020).

Natural history. *Pristimantis nebulosus* occurs in an area of evergreen montane rainforest of moderate height. Forest grows at moderate to steeply sloped terrain (Fig. 5). See also Myers and Daly (1979) for a detailed description of the area. The area has been heavily altered by different kinds of plantations (e.g. tea). Individuals were found in disturbed habitat along a narrow dirt road. Males were



Figure 5. View of the forest at the type locality of *Pristimantis nebulosus* at Abra La Divisoria, southern Cordillera Azul, Departamento Huánuco, Peru, at 1650 m a.s.l. Photo taken on 8 November 2019.

calling from a bushy area during light rain at night, one individual called from near the ground, another one was sitting on a leaf about 30 cm above the ground. The species was found in syntopy with *Pristimantis* sp. (aff. *divinae*), *P.* sp. (*lacrimosus* group), *Rhinella* sp. (*margaritifera* group), *Boana lanciformis*, *Dendropsophus aperomeus*, *Scinax garbei*, *S.* sp. (aff. *ruber*) and *Adenomera* sp.

Vocalisation. Calls were recorded on 8 November 2019, around 20:00 h, at Abra la Divisoria, Departamento Huánuco, 1650 m a.s.l. (air temperature estimated 18 °C). The recorded individual could not be observed calling, but searching at the spot of sound emission revealed an individual of *P. nebulosus*, leaving little doubt that recorded calls actually correspond to this species. The advertisement call consists of a single regularly pulsed note, repeated at a rather regular succession in short call series (Fig. 6). Call series consist of seven calls ($n = 2$), with the initial call being the longest in duration and separated from the remaining calls of the series by a longer inter-call interval with about two times the duration of other inter-call intervals. Pulses within calls (= notes) are broadly fused, but fairly countable in the oscillogram. Apart from the pulse structure, there is amplitude modulation within each call, with the greatest amplitude occurring at the beginning of the call, then decreasing to about half the initial amplitude at about one third of the call's duration and rapidly decreasing to zero at the call's end, giving the call a bell-shaped appearance in the oscillogram. Numerical parameters of 14 analysed calls (two call series) of one male are as follows: call duration (= note duration) 206–382 ms (254.1 ± 40.3 ms); inter-call

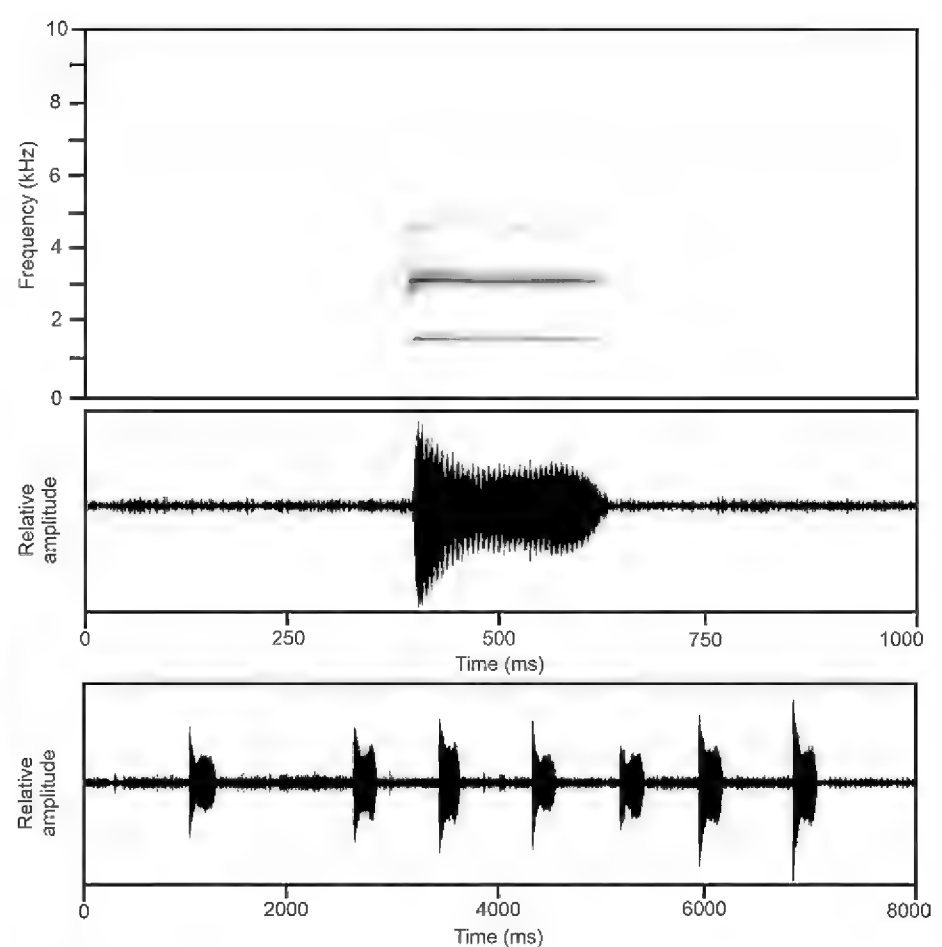


Figure 6. On top, audiospectrogram and corresponding oscillogram of one advertisement call of a topotypic *Pristimantis nebulosus* from Abra la Divisoria, Cordillera Azul, 1650 m, Peru, at 1000 ms time scale. Below, an oscillogram at 8000 ms time scale showing one complete call series consisting of 7 calls of the same individual. Recording band-pass filtered at 450–9600 Hz.

interval within call series 507–1730 ms (796.1 ± 348.8 ms); pulses per call 33–37 (35.4 ± 1.5); pulses per second 156–174 (162.8 ± 7.0); dominant frequency 3186–3359 Hz (3230 ± 57 Hz); prevalent bandwidth 1500–6600 Hz, but bands of very low call energy recognisable up to 9600 Hz. Apart from the dominant frequency band, additional frequency bands, apparently induced by the pulse rate (not harmonics; see Köhler et al. 2017), occur at approximately 1400–1900, 4400–5000, 6000–6600, and 7900–8400 Hz, respectively. Within regular call series, calls were repeated at a rate of 54–72 calls/minute. Duration of call series was 7.13 and 6.05 seconds, respectively.

Distribution. So far, *P. nebulosus* is only known from its type locality in the southern Cordillera Azul, at the border of the Departamentos Huánuco and Ucayali. However, it is very likely that the species occupies a wider range, at least along the same elevational corridor within the Cordillera Azul. Possibly, there are additional records of this species represented by unidentified or misidentified specimens in scientific collections.

Description of a new species

During our field trip in November 2019, we collected an individual of *Pristimantis* at the eastern slope of the Cordillera de Carpish in Departamento Huánuco, which we tentatively identified as *P. cf. bipunctatus*. We included a 16S sequence of this specimen in our molecular analysis. It turned out to represent a member of the *P. conspicillatus* species group, being sister to a clade containing nominal *P. bipunctatus* and *P. nebulosus*, although relationships among the samples of this subclade are not fully resolved (Fig. 4). However, this specimen exhibits considerable differentiation in the 16S gene when compared to *P. bipunctatus* (p-distances ranging from 3.3–4.1%, with the highest value of 4.1% referring to the holotype of *P. bipunctatus* KU 291638) and *P. nebulosus* (p-distance 4.1%; see Table 2). Detailed morphological examination revealed qualitative differences in character states when compared to known species in the *P. conspicillatus* group and provided further evidence for its distinctiveness. Therefore, we describe this specimen as a new species.

Pristimantis symptosus sp. nov.

<https://zoobank.org/26DBAFEE-66AD-4416-95CC-4AADB6CD23E>

Holotype. MUSM 40256 (FGZC 6207), adult male, from a point along the road 18A, approximately 5.5 km air-line distance northeast of the Carpish Tunnel (9.67744°S, 76.07313°W, 2360 m a.s.l.), Cordillera de Carpish, Departamento Huánuco, Peru, collected on 4 November 2019 by E. Castillo-Urbina, F. Glaw, and J. Köhler.

Diagnosis. A medium-sized species of the *Pristimantis conspicillatus* species group (based on molecular relationships and morphological similarity), with 27.6 mm SVL in adult male, characterised by: (1) skin on dorsum

tuberculate, with a pair of enlarged scapular warts; flanks tuberculate; throat smooth, venter weakly areolate; discoidal fold conspicuous; dorsolateral folds distinct, but low; dorsal folds absent; three prominent postrictal conical tubercles present; upper eyelid lacking tubercles and granules; posterior surfaces of thighs smooth; (2) tympanic membrane and annulus distinct, slightly higher than long, their length less than half of eye diameter; supratympanic fold long, prominent, almost straight, not covering upper tympanum or annulus; (3) head longer than wide; snout subacuminate in dorsal view, bluntly rounded in lateral profile; canthus rostralis straight in dorsal view, sharp in profile; (4) cranial crests absent; (5) dentigerous process of vomers prominent, elongate, oblique, situated posteromedial to choanae; (6) males with vocal slits, single subgular vocal sac, and nuptial asperities on dorsal surface of thenar tubercle; (7) hands with long and slender fingers, first finger equal in length to second; subarticular tubercles subconical, prominent; supernumerary tubercles absent; palmar tubercle bifid, flat; thenar tubercle prominent, elongated; terminal discs of inner two fingers enlarged and round, those of external fingers enlarged, ovate, about twice the width of digit proximal to disc; circumferential grooves conspicuous, ungual flap very slightly indented; lateral fringes on fingers absent; basal webbing between fingers absent; (8) ulnar tubercles absent; (9) tubercles on heel and tarsus absent, tarsal fold absent; (10) inner metatarsal tubercle ovate, prominent; outer metatarsal tubercle round, flat; supernumerary tubercles absent; (11) toes long and slender (FootL 54% SVL); narrow lateral fringes weakly expressed, trace of basal toe webbing present; toe V reaching distal level of penultimate subarticular tubercle of toe IV; toe V slightly longer than toe III; tips of toes rounded to slightly ovate, enlarged; circumferential grooves conspicuous; (12) in life, dorsal colouration brown to tan with dark brown chevrons and flecks on dorsum; dark brown bars on dorsal surfaces of arms and legs; dark brown interorbital bar; a pair of black spots, surrounding prominent conical scapular warts; broad black supratympanic stripe; black canthal stripe; belly cream; throat densely mottled with dark brown; ventral surfaces of thighs and shanks orange tan; posterior surface of thighs orange-brown with irregular cream spotting; plantar and palmar surfaces yellowish-brown, densely covered with dark brown mottling; iris copper, with black reticulation; posterior iris periphery pale blue; bones white; (13) advertisement call consisting of a single pulsed note of 132–186 ms duration, emitted at regular succession (see below).

Comparison. Among the now 42 recognised species in the *P. conspicillatus* species group (Padial et al. 2014, 2016; de Oliveira et al. 2017, 2020; Taucce et al. 2020), 13 species, including *P. symptosus*, exhibit dorsolateral folds (Duellman and Lehr 2009; Padial et al. 2016). Among these, *P. adistolus* mainly differs from *P. symptosus* by more prominent and longer dorsolateral folds, finger I slightly shorter than finger II, and skin on dorsal surfaces shagreen (Duellman and Hedges

2007); *P. avicuporum* mainly differs by the presence of an interocular dermal fold, fingers bearing lateral fringes, and finely shagreen dorsal skin (Duellman and Pramuk 1999; Duellman and Lehr 2009); *P. buccinator* mainly differs by continuous and prominent dorsolateral folds, an X-shaped or V-shaped dorsal fold and presence of an interorbital dermal ridge (Rodríguez 1994); *P. condor* mainly differs by larger male size (SVL 32.1–39.5 mm) and finger I distinctly longer than finger II (Lynch and Duellman 1980); *P. conspicillatus* mainly differs by finger I being distinctly longer than finger II, fingers bearing lateral keels, and dorsal skin finely shagreen (Duellman and Lehr 2009); *P. iiap* mainly differs by upper eyelid bearing small granules, skin on dorsum coarsely shagreen, basal toe webbing absent, presence of a tarsal fold, and advertisement call (Padial et al. 2016); *P. malkini* mainly differs by finger I distinctly longer than finger II and more extensive webbing between toes (Lynch 1980; Duellman and Lehr 2009); *P. meridionalis* mainly differs by finger I being shorter than finger II, dentigerous processes of vomers small and round, and fingers bearing lateral fringes (Duellman and Lehr 2009); *P. peruvianus* mainly differs by very prominent and long dorsolateral folds, finger I distinctly longer than finger II, and fingers bearing lateral fringes (Köhler 2000; Padial and De la Riva 2009); *P. skydmainos* mainly differs by more prominent and continuous dorsolateral folds, finger I being shorter than finger II, and a black mid-dorsal tubercle (Flores and Rodríguez 1997; Padial and De la Riva 2005). The closely related *P. nebulosus* mainly differs by skin on dorsum and flanks shagreen, curved supratympanic fold, relatively longer legs (TL/SVL 0.64 versus 0.60), presence of a tarsal fold, presence of basal webbing between fingers II and III, bronze-coloured iris in life, advertisement call (see below), and divergence in the mitochondrial 16S gene (p-distance 4.1%). The sister species *P. bipunctatus* is most similar to *P. symptosus* in sharing similar adult male size, finger I about equal in length to finger II, tarsus lacking a tarsal fold, fingers lacking lateral keels or fringes, a pair of enlarged scapular warts, and a similar colour pattern. However, *P. bipunctatus* (Fig. 7) differs by skin on dorsum and flanks finely shagreen (tuberculate), bluntly rounded snout in dorsal view (subacuminate), basal webbing between toes IV and V (basal webbing between all toes) (Duellman and Hedges 2005; Duellman and Lehr 2009), and considerable divergence in the mitochondrial 16S gene (p-distance 3.3–4.1%). The advertisement call of *P. bipunctatus* remains unknown. Individuals of the recently described species *P. pictus*, *P. pluvian* and *P. moa* from Amazonian Brazil may sometimes exhibit weakly developed dorsolateral folds, but all differ from the new species by shagreen dorsal skin (versus tuberculate) and advertisement call (de Oliveira et al. 2020).

Description of the holotype. An adult male, in good state of preservation (Fig. 8), with subgular vocal sac and vocal slits. Head longer than wide (HL/HW = 1.2); snout subacuminate in dorsal view, bluntly rounded in profile; nostrils oriented posterolaterally; canthus rostralis straight



Figure 7. *Pristimantis bipunctatus* (MUSM 31120; GenBank accession number KY006089) from Yanachaga-Chemillén National Park, 2290 m a.s.l., Departamento Pasco, Peru, in life: **a.** Dorsolateral view; **b.** Ventral view. Photographs by J. Moravec.

in dorsal view, sharp in profile; loreal region straight; lips not flared; upper eyelid without tubercles; cranial crests absent. Supratympanic fold prominent, long, almost straight, not covering upper tympanic membrane or annulus; tympanic membrane and annulus distinct; tympanic membrane nearly slightly higher than long, its length slightly less than half the eye diameter; three prominent postriatal tubercles, conical. Choanae not concealed by palatal shelf of the maxillary arch when roof of mouth is viewed from below; choanae large, round, separated by distance equal to six times diameter of a choana; dentigerous process of vomers prominent, elongate, not in contact, oblique, situated posteromedial to choanae, bearing vomerine teeth; tongue removed for tissue sample; vocal sac subgular, vocal slits placed posterolaterally. Skin on dorsum tuberculate, with a pair of prominent conical scapular warts; dorsal surfaces of hind limbs tuberculate, dorsal surfaces of forearms and flanks tuberculate; skin on throat and chest smooth, that on belly areolate; occipital folds absent; dorsolateral folds distinct, but low; discoidal fold conspicuous. Arm without ulnar tubercles; palmar tubercle bifid, flat, equal in size to elongate thenar tubercle; supernumerary tubercles absent; subarticular tubercles prominent, subconical; fingers long and slender; finger tips enlarged, rounded on inner two fingers, on two outer fingers ovate, their width about twice the width of digit proximal to disc; circumferential grooves conspicuous, ungual flap very slightly indented on outer fingers; lateral fringes and keels on fingers absent; basal webbing between fingers absent; relative length of fingers $III > IV > II = I$; nuptial pad present on dorsal surface of thumb. Toes long and slender (FootL 54% of SVL); heel and tarsus lacking tubercles; tarsal fold absent; inner metatarsal tubercle ovate, prominent, larger than outer; outer metatarsal tubercle round, flat, about one third the size of outer; tarsal fold absent; supernumerary tubercles absent; subarticular tubercles prominent, subconical; narrow lateral fringes on toes weakly expressed, traces of

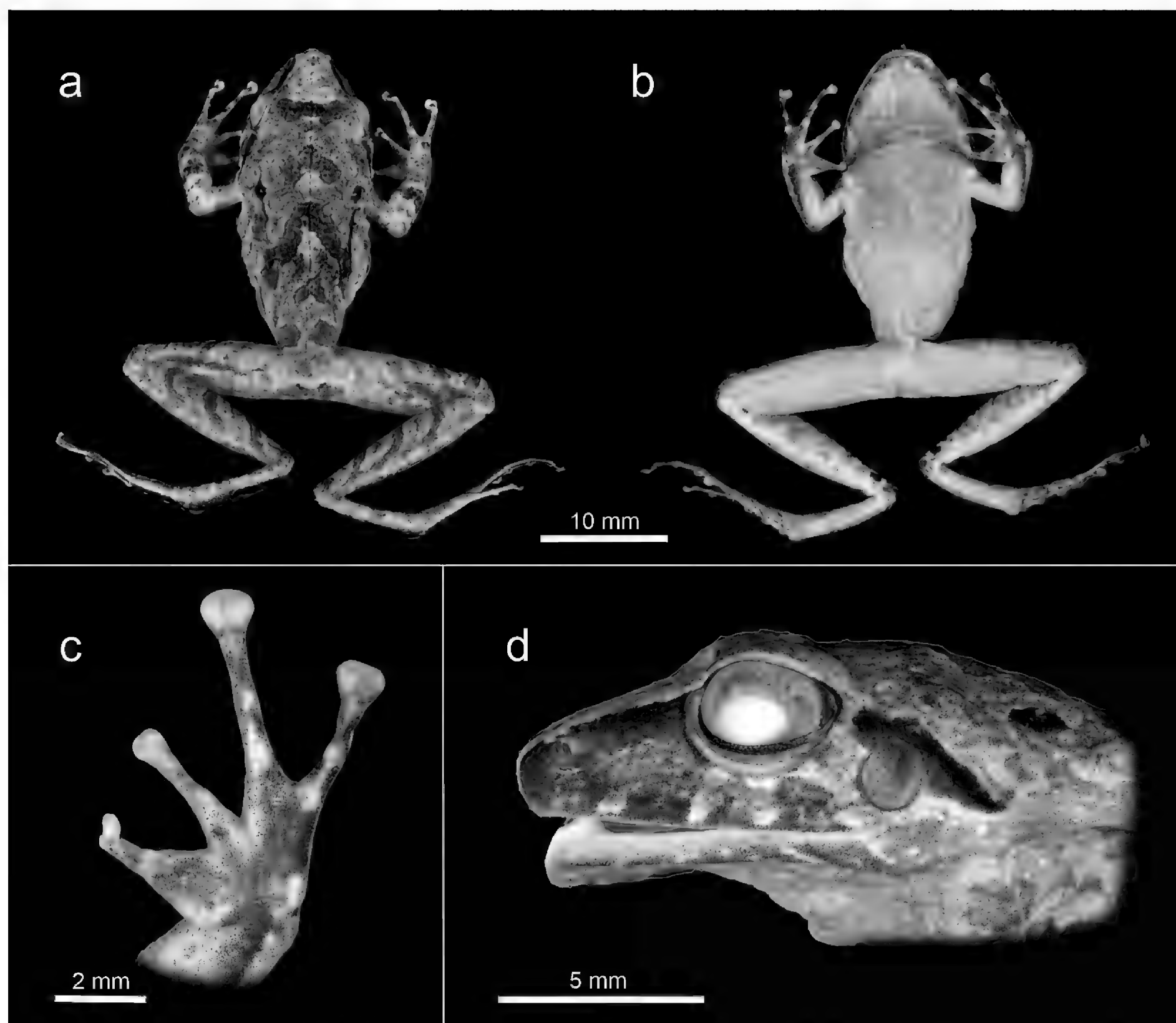


Figure 8. Preserved male holotype of *Pristimantis symptosus* sp. nov. (MUSM 40256, FGZC 6207) from the Cordillera de Carpish, 2360 m a.s.l., in **a.** dorsal, and **b.** ventral views; **c.** Palmar surface of left hand; **d.** Head in lateral profile (mirrored). Note prominent scapular tubercle surrounded by black spot.

basal toe webbing present; toe tips enlarged, rounded to slightly ovate, their width about 1.5 times the width of toe proximal to disc; circumferential grooves conspicuous; relative length of toes $IV > V > III > II > I$; toe V reaching distal level of penultimate subarticular tubercle of toe IV. Tibiotarsal articulation reaches slightly beyond tip of snout when hind limb flexed parallel to axis of body; heels overlapping when hind limbs flexed perpendicular to axis of body.

Measurements (in mm): SVL 27.6; TL 16.8; HL 11.9; HW 9.9; IOD 3.4; ED 3.9; TD 1.7; E–N 3.1; HandL 7.9; FootL 14.8.

In life (Fig. 9), dorsal ground colour brown to tan, with dark brown chevrons and irregular dark brown blotches on dorsum, most of them finely outlined with cream; dark brown bars on dorsal surfaces of arms and legs; dark brown interorbital bar, extending to upper eyelids; a pair of black spots surrounding prominent scapular warts; broad black supratympanic stripe; broad blackish canthal

stripe; lips dark brown to black, barred with cream; flanks brown with irregular dark brown markings; belly cream; throat and chest orange-brown with dense black mottling; ventral surfaces of thighs and shanks orange tan; posterior surface of thighs orange-brown with irregular cream spotting; plantar and palmar surfaces yellowish-brown, densely covered with dark brown mottling; iris copper, with black reticulation; posterior iris periphery pale blue; bones white. After two years in preservative (Fig. 7), the general colour pattern remains the same as in life. Brown ground colouration turned to greyish-tan, with brown flecks, bars and chevrons; black colour of canthal and supratympanic stripes faded to anthracite; chest and ventral surfaces of thighs yellowish-cream; belly cream; throat cream with grey mottling.

Natural history. The forest at the type locality constitutes upper montane rainforest at the transition zone to cloud forest, growing on steep slopes (Fig. 10), with trees not exceeding 20 m height (Jiménez and Pacheco 2016).

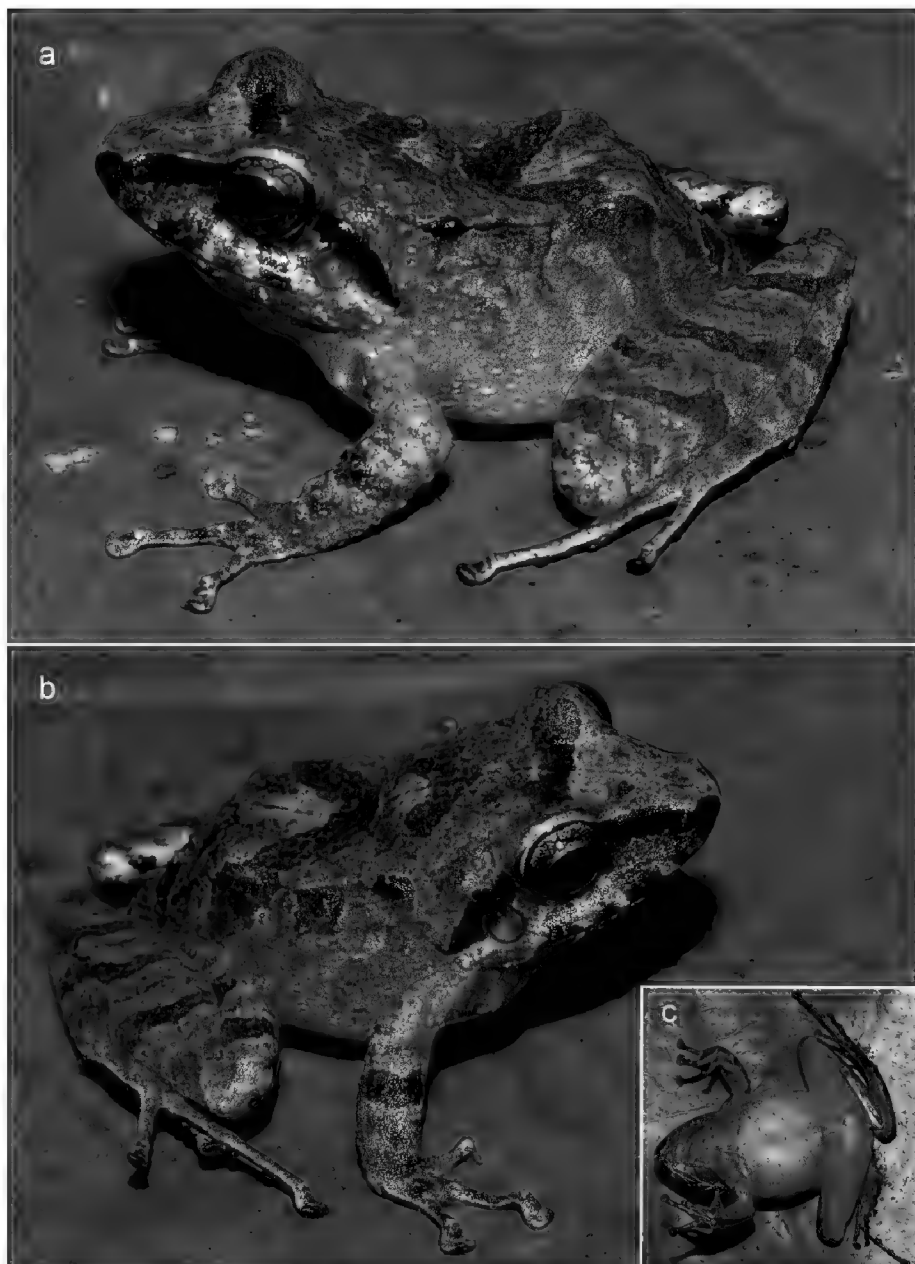


Figure 9. Male holotype of *Pristimantis symptosus* sp. nov. (MUSM 40256, FGZC 6207) from the Cordillera de Carpish, 2360 m a.s.l., in life: **a.** Dorsolateral view of left body side; **b.** Dorsolateral view of right body side; **c.** Ventral view. Note tuberculate skin on dorsum, flanks and forearm.

Males were calling from a low position in shrub vegetation along the road during a foggy night and light rain. The holotype was sitting on a leaf approximately 25 cm above the ground. *Pristimantis* sp. (*lacrimosus* group) and *P.* sp. (aff. *rhacodermus*) were found at nearby sites. Nothing else is known.

Vocalisation. Calls were recorded at the type locality of *P. symptosus* on 4 November 2019 (estimated air temperature 16 °C). Calling males were easily disturbed and stopped calling when being approached at distances of less than 10 m. Therefore, recordings are of poor quality, as the recorded individual was calling at considerable distance (estimated > 15 m) and there is considerable background noise in the recording. A call voucher could not be collected, but the male holotype was caught at a spot from where the same type of call was emitted (although the individual could not be directly observed calling), making it very probable that the calls described here belong to *P. symptosus*. Although the poor recording quality hampers access to some call parameters (i.e. pulse structure and exact rate), the call can be described as follows. The advertisement call consists of a single, short, pulsed note, repeated at regular succession (Fig. 11). Calls (= notes) exhibit a very slight upward frequency modulation and are clearly pulsed, although the poor recording quality



Figure 10. View of the forest along the road 18A close to the type locality of *Pristimantis symptosus* sp. nov. in the Cordillera de Carpish, Departamento Huánuco, Peru, at approximately 2450 m a.s.l. Photo taken on 4 November 2019.

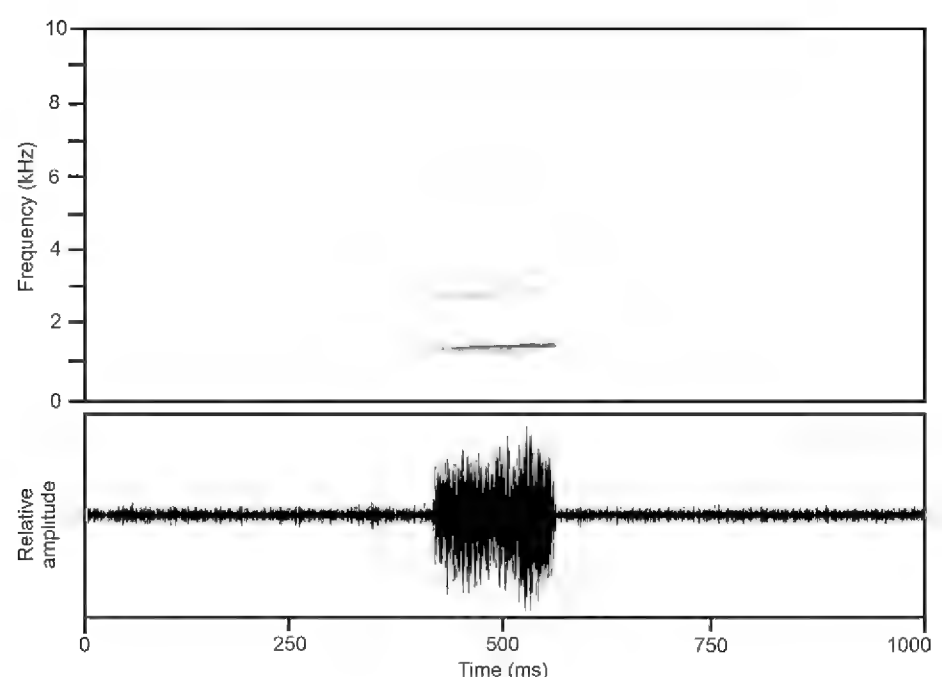


Figure 11. Audiospectrogram and corresponding oscillogram of one advertisement call allocated to *Pristimantis symptosus* sp. nov. from Cordillera de Carpish, 2360 m a.s.l., Peru, at 1000 ms time scale. Recording band-pass filtered at 850–6500 Hz.

prevents any reliable counting of pulses; however, it can be estimated from some sections of the calls that pulse rate is greater than 200 pulses/second. Numerical parameters of 23 analysed calls are as follows: call duration (= note duration) 132–186 ms (151.3 ± 14.1 ms); inter-call interval 804–1041 ms (882.3 ± 79.5 ms); dominant frequency 1442–1507 Hz (1468 ± 25 Hz), with a second

peak in call energy of similar intensity at around 2800–2930 Hz; prevalent bandwidth 1200–4000 Hz; calls were repeated at a rate of 52–63 call/minute.

Distribution. *Pristimantis symptosus* is only known from its type locality and possibly endemic to the Cordillera de Carpish.

Etymology. The specific epithet is a Latinised adjective derived from the Greek σύμπτωσις (symptosi) meaning ‘coincidence’. It refers to the fact that we only discovered the new species by coincidence on an unplanned return to the Cordillera de Carpish after forgetting part of our expedition gear there.

Discussion

As mentioned in the Introduction, the status of *Pristimantis nebulosus* (Henle, 1992) has long been enigmatic. The main reasons for this were an insufficient original species description (Henle 1992) and a poor state of preservation of the holotype. Without any newly collected topotypic specimens, we would have been unable to evaluate the status of this taxon by examination of the holotype alone (see Duellman and Lehr 2009). A superficial first examination of the type specimen gave the impression of certain differences in body proportions when compared to newly collected topotypic specimens. Only a very detailed specimen-to-specimen comparison of the holotype and topotypes, taking into account certain artifacts and damage of the type specimen, finally resulted in a convincing allocation of this nominal taxon to a living population of *Pristimantis*. Although we are completely convinced, given the congruence in morphological traits, that our collected topotypes are conspecific with the holotype of *P. nebulosus*, further evidence could be achieved by obtaining molecular genetic data from the holotype. We tried to extract and amplify DNA from a small piece of thigh muscle of the holotype using standard protocols, but this approach unfortunately failed. The established, but challenging and costly method of target-enriched sequencing of archival DNA from old specimens (e.g. Scherz et al. 2020; Vences et al. 2021) would currently be an option to obtain sequences for the holotype of *P. nebulosus*, but without any guarantee of success. However, this method remains available as a promising future option for many old type specimens of species with uncertain status currently placed in the species-rich genus *Pristimantis*.

In recent decades, the Cordillera de Carpish in central Peru has been the place of discovery of several new anurans and seems to hold a remarkable diversity of endemic species (see Lehr and Rodríguez 2017 for a summary). Our molecular analysis indicated the presence of a new candidate species in the *Pristimantis conspicillatus* species group from the Cordillera de Carpish being sister to *P. bipunctatus*, but considerably divergent from it. The herein described *Pristimantis symptosus* differs from the holotype of *P. bipunctatus* (KU 291638, EF493702) originating from east of Oxapampa (at the border of

Yanachaga-Chemillén National Park) by 4.1% divergence in the 16S fragment. The divergence between nominal *P. bipunctatus* samples from Yanachaga-Chemillén and Pui Pui Forest further south is pronounced (3.7%), indicating a possible complex of species hidden under the name *P. bipunctatus*. Remarkably, the genetic distance of *P. symptosus* to *P. bipunctatus* samples originating from the geographically more distant Pui Pui Forest in the south is lower (3.3%) than the genetic distance to *P. bipunctatus* samples from the geographically closer Yanachaga-Chemillén National Park (3.9–4.1%; see also Fig. 12). *Pristimantis symptosus* is further divergent from the herein identified and closely related, but morphologically different *P. nebulosus* by a genetic distance of 4.1%.

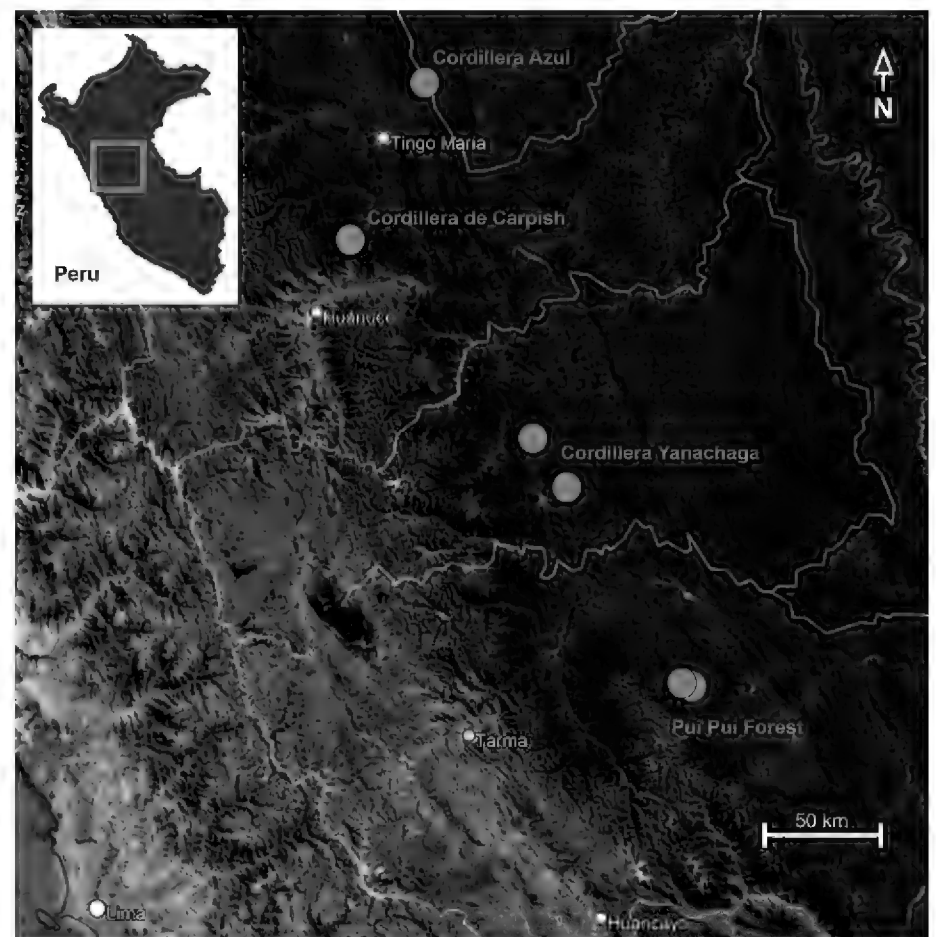


Figure 12. Satellite image of central Peru (from GoogleEarth) indicating localities of *Pristimantis* samples included in our molecular analysis: *P. nebulosus* (light blue), *P. symptosus* sp. nov. (yellow) and *P. bipunctatus* (red).

In fact, providing unambiguous evidence for the existence of a new species is challenging, when based on a single specimen. Castroviejo-Fisher et al. (2011) discussed the problems of limited sample size in anuran taxonomy. However, in certain cases, the description of so-called singleton species (sensu Lim et al. 2012) is justified and warranted (see Köhler and Padial 2016; Scherz 2020). The study of specimens of *Pristimantis* over the past decades indicates that certain characters, such as relative finger length and dorsal skin texture, are barely variable within species, even across specimens of different sex and age (e.g. Duellman and Lehr 2009). Here, we provided multiple lines of evidence for the distinctiveness of the new species including genetic differentiation, qualitative differences in morphological characters and advertisement call. Molecular analyses clearly placed the new species in the *P. conspicillatus* species group, but with considerable differentiation to other species of the group (p-distances

3.3–17.8% to all other species). The combination of tubercular dorsal skin, areolate skin on venter, presence of dorsolateral folds, first finger about equal in length to second, basal webbing between all toes, and a copper iris in life distinguishes *P. symptosus* morphologically from all other recognised species in the *P. conspicillatus* group. Furthermore, the advertisement call differs from all other calls known for species in the *P. conspicillatus* group (e.g. Köhler 2000; Padial and De la Riva 2009; Padial et al. 2016). However, advertisement calls have been described only for a fraction of species in this group and molecular data for some species allocated to the group are still lacking. The unknown call of *P. bipunctatus* and several related species argue for future bioacoustic investigations of this taxonomically challenging group of *Pristimantis*. Nevertheless, given the combination of all lines of evidence as described above, we think that the description of this singleton species is justified and warranted. Of course, aspects such as intra-specific variability, sexual dimorphism and natural history will remain unknown until further research finds additional individuals of *P. symptosus*, but the data provided with this species description provide the taxonomic foundation for such future studies.

Given the considerable genetic differentiation among nominal *P. bipunctatus* from different localities mentioned above, the close relationships of *P. symptosus* and *P. nebulosus* to these and the presence of rather similar, almost cryptic species like *P. adiaxolus* (see Duellman and Hedges 2007; Duellman and Lehr 2009), we hypothesise that the eastern montane forests of central Peru may still hold a variety of undescribed species in the *P. conspicillatus* group (with *P. bipunctatus* from Pui Pui Forest representing one potentially undescribed candidate species), all very similar in general appearance.

Although an investigation of other groups of *Pristimantis* was beyond the aim of this study, we herein document considerable genetic differentiation within the *P. danae* species group. As already revealed by former studies (Moravec et al. 2020), there is marked genetic differentiation in the 16S gene fragment among samples considered to represent *P. reichlei* (Fig. 4). The inclusion of our samples tentatively identified as *P. cf. reichlei* from Panguana (ca. 230–260 m a.s.l.), Departamento Huánuco, showed these samples to be highly divergent from and, perhaps, not even the phylogenetically closest relatives of *P. reichlei*, with p-distances of 8.7% to topotypic *P. reichlei* from Bolivia (Los Guácharos, Departamento Cochabamba; Padial and De la Riva 2009), and 8.1–8.5% to other *P. reichlei* from different Peruvian localities. We, therefore, flag the respective population occurring at Panguana as a new candidate species whose status will be investigated in a forthcoming study.

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Appendix 1

Table A1. List of *Pristimantis* samples plus *Yunganastes pluvicanorus* (outgroup) used for the molecular analysis of the 16S gene fragment, including voucher number, GenBank accession number and locality. Order of samples are as they appear in Figure 4 from top to bottom. Accession numbers in bold refer to newly obtained sequences for this study.

Species	Voucher specimen	GenBank accession number	Locality
<i>P. bipunctatus</i>	NMP6V/75062	KY006090	Peru: Junín: Pui Pui Forest, 1700 m a.s.l.
<i>P. bipunctatus</i>	MUSM 31179	KY594758	Peru: Junín: Pui Pui Forest, 1615 m a.s.l.
<i>P. bipunctatus</i>	KU 291638	EF493702	Peru: Pasco: East of Oxapampa, 2120 m a.s.l.
<i>P. bipunctatus</i>	MUSM 31120	KY006089	Peru: Pasco: PN Yanachaga-Chemillén, 2290 m a.s.l.
<i>P. nebulosus</i>	MUSM 40299, FGZC 6254	ON710982	Peru: Huánuco: Abra La Divisoria, 1650 m a.s.l.
<i>P. nebulosus</i>	MUSM 40298, FGZC 6253	ON710981	Peru: Huánuco: Abra La Divisoria, 1650 m a.s.l.
<i>P. nebulosus</i>	MUSM 40326, FGZC 6281	ON710983	Peru: Huánuco: Abra La Divisoria, 1650 m a.s.l.
<i>P. symptosus</i>	MUSM 40256, FGZC 6207	ON710980	Peru: Huánuco: Cordillera de Carpish, 2360 m a.s.l.
<i>P. cf. skydmainos</i>	ZSM 1980/2008	ON710985	Peru: Huánuco: Panguana
<i>P. skydmainos</i>	MHNSM 10071	EF493393	Peru
<i>P. gutturalis</i>	577 PG	JN691313	French Guiana: Mitaraka
<i>P. incertus</i>	–	EU186650	Trinidad and Tobago: Tobago: King’s Bay Reservoir
<i>P. zeuctotylus</i>	209 AF	JN691259	French Guiana: Angouleme
<i>P. vilarsi</i>	AJC 3944	KP149278	Colombia: Meta: Finca Los Almendros
<i>P. fenestratus</i>	CFBHT 05708	KU495458	Brazil: Amazonas: Manaus
<i>P. fenestratus</i>	INPAH 20931	KY712673	Brazil: Amazonas: Borba
<i>P. koehleri</i>	MNCN 42990	EU192278	Bolivia: Santa Cruz: Angostura-Samaipata road
<i>P. samaipatae</i>	MNCN 42987	EU192292	Bolivia: Santa Cruz: Angostura-Samaipata road

Species	Voucher specimen	GenBank accession number	Locality
<i>P. latro</i>	LZATM 467	KX242519	Brazil: Para: Anapu
<i>P. chiastonotus</i>	162AF	EU201061	Suriname: Brownsberg
<i>P. vinhai</i>	UFMG 19856	MN954211	Brazil: Bahia: PN Serra das Lontras
<i>P. palodutrai</i>	MNRJ 40299	JX267360	Brazil: Bahia: Canavieiras
<i>P. ramagii</i>	MNRJ 50249	JX267319	Brazil: Alagoas: Murici
<i>P. lymani</i>	KU 218019	EF493392	Ecuador: Loja: 3.9 km E Loja
<i>P. achatinus</i>	QCAZ 62884	MK829664	Ecuador: S. D. de los Tsáchilas: Valle Hermoso
<i>P. malkini</i>	QCAZ 25544	MH516185	Ecuador: Napo: Jatun Sacha
<i>P. malkini</i>	QCAZ 28296	EU186663	Ecuador: Sucumbios: Monte Tour
<i>P. citriogaster</i>	KU 212278	EF493700	Peru: San Martín: 14 km NE Tarapoto
<i>P. condor</i>	KU 217857	EF493701	Ecuador: Morona Santiago: N of Galaquiza
<i>P. conspicillatus</i>	QCAZ 28448	EF493529	Ecuador: Sucumbios
<i>P. buccinator</i>	MNCN-DNA 9504	EU712630	Peru: Cuzco: Blanquillo
<i>P. buccinator</i>	MUSM 33269	KY652650	Peru: Madre de Dios: Los Amigos Biological Station
<i>P. rupicola</i>	UFMG 20568	MN954206	Brazil: Bahia: Serra do Buriti do Ouro
<i>P. gageae</i>	KRL 1079	FJ784461	Panama: El Copé: PN Omar Torrijos
<i>P. pulchridormientes</i>	CORBIDI 15566	KX664107	Peru: Huánuco: PN Tingo Maria
<i>P. pluvialis</i>	CORBIDI 11862	KX155577	Peru: Cuzco: Kosnipata near Chontacha
<i>P. lacrimosus</i>	QCAZ 40261	MT636524	Ecuador: Pastaza
<i>P. bromeliaceus</i>	QCAZ16699	MT636527	Ecuador: Morona Santiago
<i>P. lanthanites</i>	KU 222001	EF493695	Peru: Loreto: San Jacinto
<i>P. colomai</i>	QCAZ 17101	EF493354	Ecuador: Esmeraldas: Alto Tambo
<i>P. toftae</i>	KU 215493	EF493353	Peru: Madre de Dios: 15 km E Puerto Maldonado
<i>P. rhabdolaemus</i>	MNCN 43036	EU192257	Bolivia: Santa Cruz: La Yunga de Mairana
<i>P. danae</i>	MNCN 44234	EU192270	Peru: Cuzco: Unión, Kosnipata valley
<i>P. danae</i>	MNKA 7273	EU192265	Bolivia: La Paz: Serranía Bella Vista
<i>P. reichlei</i>	CORBIDI 16219	KY652657	Peru: Cuzco: Kosnipata valley
<i>P. reichlei</i>	MNCN 43249	EU192288	Peru: Cuzco: 5 km from San Lorenzo to Qince Mil
<i>P. reichlei</i>	MNCN 43012	EU192287	Bolivia: Cochabamba: Los Guácharos
<i>P. reichlei</i>	IDLR 4779	EU192285	Peru: Puno: entre Puerto Leguia y San Gabán
<i>P. cf. reichlei</i>	ZSM 1985/2008	ON710986	Peru: Huánuco: Panguana
<i>P. cf. reichlei</i>	ZSM 1986/2008	ON710987	Peru: Huánuco: Panguana
<i>P. cf. reichlei</i>	FGZC 5342	ON710989	Peru: Huánuco: Panguana
<i>P. cf. reichlei</i>	FGZC 6334	ON710984	Peru: Huánuco: Panguana
<i>P. cf. reichlei</i>	FGZC 3300	ON710988	Peru: Huánuco: Panguana
<i>Y. pluvicanorus</i>	MNCN-DNA 6004	EU192247	Bolivia: Cochabamba: Penstock bridge